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RESEARCH ARTICLE

A REVIEW OF BREAKING SEED DORMANCY IN HAWTHORNS (*Crataegus* spp.)

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Abstract

Propagation of hawthorns (*Crataegus* spp.) is difficult, and vegetative methods are not responsive in a sufficient manner. So, seed propagation is prevalently used to propagate hawthorns. However, hard endocarp and embryo dormancy restrict the germination of hawthorns. To overcome these restrictions, many treatments have been tested in the researches. Some of them were magnificent and others ineffective. It has been resolved that warm and cold stratification are the best for breaking dormancy in seeds of hawthorns. Thus far, no substitution has been found for warm and cold stratification treatment to release the seeds of hawthorns from dormancy, from the conventional point of view. Besides, combination with other factors may reduce the period and temperature degrees of warm and cold stratification, for instance, species, eliminating endocarp, treatment with growth regulators, and chemical scarification. In this review, the results of methods will be referred to that used in the studies to break seed dormancy in hawthorns.

Introduction:-

Hawthorn (*Crataegus* spp.) is belonging to the Rosacea family, of which 200 different species have been recognized under this genus in Northern hemisphere (Özyurt et al., 2019). The species of this genus are deciduous and have either shrub or tree nature (Mamıkoğlu, 2007). Despite the nutritional value which they provide to humans and wildlife, they are used to take advantage in the area of medicine and pharmacology (Gokturk et al., 2017). Recently, the effect of preparations from the leaves and flowers of hawthorns has been focused on by many researchers on cardiovascular disease (CVD), and positive results were reached if they were not consumed in long term (Tassell et al., 2010). Besides, hawthorns have deep and pile root systems and resist harsh climates, thus could be employed as fencing, wind breaking, and protecting lands from erosion (Kovář et al.,1996; McAdam et al., 1996). Also, successful results were obtained when hawthorn was applied as a rootstock for pear, apple, and loquat in order to water-saving because they are highly tolerable to drought (Qrunfleh, 1993; Polat, 2022).

The methods of propagation of hawthorns are seed propagation and vegetative methods (cutting, grafting, and budding). Because of scarce rooting, cutting propagation is not recommended. So, hawthorns are prevalently propagated by seeds (Ahmadloo et al., 2015b). Propagation of hawthorns by seed is challenging since their seeds do not readily germinate on shedding if it is not exposed to special treatments, and may remain for 2–3 years in a dormant state (Bujarska-Borkowska, 2006). Exogenous and endogenous dormancy, which is so-called double dormancy, occur in seeds of hawthorns (Ahmadloo et al., 2017). More precisely, Phipps (1998) referred that the *Crataegus* spp. are endemic to warm temperate climates have only endocarp dormancy. In contrast, the ones that originate in the colder

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regions exhibit embryo dormancy together with endocarp dormancy. In light of these, many pre-sowing treatments have been applied to the seeds of *Crataegus* spp. aimed to overcome seed dormancy and facilitate germination. In this review, the results of the treatments studied in many kinds of researches will be discussed.

Combination of warm and cold stratification

Seed dormancy in *Crataegus* is a combinational state due to having hard endocarp and physiological barriers (Bujarska-Borkowska, 2007). Seed germination takes a long time, maybe 2 years or more. This has forced the growers to employ many pre-treatments for the seeds of this genus (Bujarska-Borkowska, 2002). Because *Cratageus* seeds have physiological embryo dormancy, cold stratification was applied to the seeds before sowing (Brinkman,1974). Cold stratification is important to elevate gibberellin (GA_3) synthesis, which activates embryo growth to penetrate seed coat, at the same time reduces abscisic acid (ABA) which is notoriously known for its effect on inhibiting seed germination (Sharma and Sharma, 2010). However, Bärtels (1982) showed that cold stratification alone at 3–5°C for 180 days induced germination in the next spring of some of the seeds, but most of them stayed in a dormant state for 1 or 2 years. Further, Qrunfleh (1991) reported that stratified seed of *C. azarollus* at 5 °C for (0, 20, 40, 60, 80,100, and 120 days) demonstrated a steep decline in ABA after 20 days and a slow gradual reduction in the followings. He found that this decline in ABA correlated with germination increase at 100 and 120 days as well, but not at a high rate (23 to 24%). So, he suggested prolonging the stratification period for more than 120 days. Besides, the effect of cold stratification may be variable depending on the species. Cold stratification was effective to increase germination percentage in *C. monogyna* Jacq. from 0% to 76% at 4 °C for 4 months (Kheloufi et al., 2019). The expertise to increase cold stratification efficiency, warm stratification regime for a while before cold stratification has been adopted. The mechanism of action of warm stratification followed by cold stratification on seed may be due to during warm stratification microbial decomposition of hard seed coats occur and embryo dormancy may break as a result of the following cold stratification (Baskin et al., 2002). Also, warm stratification helps the embryo's full maturation (after ripening) in the species whose seeds are dispersed while the embryo doesn't reach full maturity (Doody and O'Reilly, 2011). Three species of hawthorn were subjected to warn and cold stratification (Downy hawthorn, Arnold hawthorn, and Fireberry hawthorn). It was detected that the species differently responded to the warm stratification. The least warm stratification period was 60 days at 18 to 22 °C for Downy hawthorn and Arnold hawthorn followed by 120 days of cold stratification at 2 to 4 °C, but 90 to 120 days of warm stratification required for Fireberry hawthorn accompanied by 120 days of cold stratification (Morgenson, 2000). Furthermore, cyclic alternation in temperature and period of exposure to warm stratification was potent for breaking dormancy in hawthorns. Bujarska-Borkowska (2002) concluded three best-alternated regimes of warm stratification for 16 weeks [25°C, 20~30°C (16+8hrs/day), and 20~30°C (24+24 hrs)] to break dormancy in seeds of *C. monogyna* in advance of cold stratification for 14 –18 weeks at 3°C, the warm and cold stratifications were in a moist medium of sand and peat 1:1 v/v. Similar results were obtained with the seed stratification of *C. laevigata* in a moist medium, and with the seeds of *C. submollis* without medium at almost the same regimes referred to above (Bujarska-Borkowska, 2006; 2007).

On the other hand, a combination of other factors with warm and cold stratifications will increase the competence of the stratifications. As aforementioned warm stratification is used to overcome the barriers due to the hard seed coat. One study by Persson et al. (2006) was done on seeds of *C. monogyna* emphasized that the seeds with endocarp required 112 days of warm stratification and then cold stratification at 5 °C, but in the seed without endocarp, germination was seen without receiving any warm stratification before cold stratification. Moreover, the germination of seeds without endocarp was higher than of those with endocarp at all durations of warm stratification. Additionally, Fatemeh et al. (2014) achieved the best germination percentage in seeds of *C. pseudoheterophylla* when they co-inoculated the seed with plant growth promoting rhizobacteria (PGPRs) and then stratified in alternate temperature regime, three times for 1 month at 4°C, then 2 weeks at 23°C. Further, soaking the seeds of *C. monogyna*in 500 mg L^{-1}polystimulins (PS A6-auxin + PS K-cytokine) for 24 hours prior to 45 days of warm stratification at 25 °C then 180 days of cold stratification at 4 °C enhanced germination percentage (Ertekin, 2017). Similarly, Ahmadloo et al. (2017) utilized GA_3 at 3000 mg L^{-1} to the seeds of *C. pseudoheterophylla* with or without endocarp, GA_3 at 3000 mg L^{-1} raised germination percentage to 59.7% in the seeds without endocarp after subjecting to the cyclic alternate temperature of warm and cold stratification for three times (1 month at 4°C, then 2 weeks at 23°C, the third cycle just for 1 month at 4 °C).

Relationship between chemical scarification and stratification

Hawthorns are known for a thick and hard endocarp. Hence, to lessen the rigidity of the endocarp, chemical scarification of the seed endocarp has been employed on the hawthorns. Different endocarp thicknesses in hawthorns were measured based on species. Washington hawthorn is a species that has a thin endocarp and can germinate without chemical scarification, in contrast others have a thick endocarp which may reach (up to 5 mm) (Bonner, 2008). Chemical scarification increases water and gas permeability into the seed and eliminate the physical impedes around the embryo via corrosion of the seed coat (Yahyaoglu et al., 2006). The length of exposure and concentration of the corrosive chemicals depends on the species. Accurately, Gokturk et al. (2017) showed in their study the differences in the seed coat thickness among some species of *Cataergus*, along with the effect of sulphuric acid on the degree of reducing seed coat thickness according to the species. So, they found the thinnest part (0.82 mm) in *C. orientalis* but (1.63 mm) in *C. pseudoheterophylla.* One-hour treatment with concentrated sulphuric acid declined 4.68% of the diameter, and five hours' treatment scarified 10.04% of the diameter. *C. orientalis* was most affected by the acid scarification, whereas *C. pontica* was least affected. Additionally, St John (1982) summarized that treating seeds of *C. monogyna* for 30 min to 2 h in hot sulphuric acid was satisfactory, and up to 4 h for *C. crus-galli* and *C. prunifolia*. He further described that 2 h treatment of *C. coccinea* seeds then warm and cold stratification for 4 weeks and 12 weeks, respectively gave 80% germination. Also, immersion seeds of *C. monogyna* and *C. azarolus* in H_2SO_4 for 6 h + ash solution for 144 h increased germination in *C. azarolus* (64.98%), more than in *C. monogyna* (24.41%) (Göktürk and Yildirim, 2020). Soaking seeds of *C. pseudoheterophylla* in 1% hydrogen peroxide for 120 min resulted in (26.7%) germination in combination with three alternate cold-warm stratifications (1 month at 4°C and 2 weeks at 23°C) for three times (Ahmadloo et al., 2015a). In addition, Radsarian et al., (2017) reported hot water –sulphuric acid (1 hour in 85 °C water + 15 min in 98% sulphuric acid) as the best treatment for seeds of *C. pontica*. While, the seeds of *C. turkestanica* required one hour of treatment with sulphuric acid (98%) before120 days of cold stratification (Hematifar et al., 2018). Moreover, seed treatment of *C. pseudoheterophylla* with sulphuric acid at (96%) for 120 min plus alternate stratification for three rounds (1 month at 4 °C and 2 weeks at 23 °C) gave the best (55%) germination (Ahmadloo et al., 2016). Chemical scarification doesn't become an alternative to warm stratification preceding cold stratification. It was resolved that employing sulphuric acid before cold stratification had unfavourable consequences on emergence, but using it in advance of warm stratification and then cold stratification shortened the warm stratification period and improved germination percentage (Bujarska-Borkowska, 2002; 2006; 2007).Gokturk et al. (2021) indicated that storing the seeds of *C. azarolus*for 10 months at 15 °C followed by treating them with sulphuric acid for 3 hours, ash solution for 4 days, and sowing in August gave the best results. They also acquired 6.15%, 10.47%, and 11.51% of corrosion in the diameter of the seeds which were treated with sulphuric acid for 1, 3, and 6 hours, respectively. Besides, sulphuric acid application together with cold stratification was not as effective as ash solution and cold stratification for the seeds of *C. orientalis* and *C. pontica*, the germination barriers in *C. pontica* were lesser than of *C. orientalis* (Baba, 2017). Göktürk and Yılmaz (2015) declared the ineffectiveness of pre-treatments of sulphuric acid, nitric acid, and citric acid on seed germination of *C. orientalis* paal. Ex. M. Bieb, after they were sown in September and August.

Role of sowing time on germination of *Crataegus* spp.

The sowing time of the seeds sometimes contributesto seed germination of the plants because may meet the environmental needs to germinate the seeds, or gives the opportunities to overcome the dormancy of the seeds as a result of the fluctuations occur in the temperature (Pipinis et al., 2018). In relation to sowing time, seeds of *C. azarolus* were sown from December until June, but no germination was observed (Mahd et al., 2017). Whereas, sowing time could be influential for *Crataegus* if they are pre-treated. Göktürk and Yılmaz (2015) found in the seeds of *C. orientalis* paal. Ex. M. Bieb that soaking in water prior to sowing in August gave the maximum germination percentage rather than in September.

In vitro germination

Embryo culture is a substitute for eliminating the problem faced germination process of the seeds in a short time, which is so-called embryo rescue. Culturing mature embryos in synthetic media gets rid of the seed germinating inhibitors and dormancy, and it just needs a simple medium containing agar, sugar, and minerals (Fathi and Jahani, 2012). Regarding, Dinçer et al. (2022) achieved 100% germination of *C. monogyna* Jacq. seeds on a Murashige & Skoog (MS) medium. Similarly, germination of *C. azarolus* seed reached 77% in 7 – 20 days in a solid medium of MS without growth regulators (Al-Hadeedy and Al-Mallah, 2010).

Conclusion:-

Based on the results of researches have been conducted on the seeds of hawthorns, it can be concluded that cold stratification alone is not enough to break dormancy in hawthorns. While, warm stratification for a while before cold stratification is outstanding to release from dormancy. Species definitely determine the period of warm-cold stratification. Also, the cyclic alternate regime of warm and cold stratification is competent to obtain better results. Besides, using specific treatments in advance to warm and cold stratification have favorable consequences on germination in *Cataergus* spp., such as removing the endocarp, and applying growth regulators etc. Furthermore, chemical scarification shortens the required period of warm stratification before cold stratification. Whereas, sowing time didn't have promising results, but in combination with other treatments prior to sowing may be effective. The embryo culture of *Cataergus* spp. is an encouraging alternative method that successfully induced germination in a short time.

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